Author’s response to reviews

Title: Anaphase-promoting complex/cyclosome protein Cdc27 is a target for curcumin-induced cell cycle arrest and apoptosis

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Author’s response to reviews: see over
Anaphase-promoting complex/cyclosome protein Cdc27 is a target for curcumin-induced cell cycle arrest and apoptosis by Lee and Langhans

We thank the reviewers for their thoughtful comments on our manuscript and we feel that addressing the comments has significantly improved the quality of the manuscript. All changes are underlined in the revised version.

Reviewer: Sophie Barille-Nion

1. Curcumin treatment clearly triggered a G2/M cell-cycle block and apoptotic cell death. Do the authors check that apoptosis occurred during curcumin-induced mitotic arrest?

Treatment with 10 µM curcumin for 24 hours arrests about 75% of the cells in G2/M. Under these conditions cleaved caspase-3 as well as cleaved PARP were detected. These findings were published in a previous manuscript from our lab (Lee et al., 2011, BMC Cancer 11:144).

2. Could the cdc27 depletion mimic curcumin treatment in DAOY cells? This experiment would directly link impact of curcumin on cell death to its capacity to crosslink cdc27.

As requested by this reviewer we knocked down Cdc27 from DAOY cells using an RNAi approach. However, reduction of Cdc27 levels by more than 90% did not induce cell death suggesting that the cross-linking of Cdc27 by curcumin itself could induce apoptosis. While we agree that showing a direct link between Cdc27 crosslinking and cell death would be desirable we feel that this is beyond the scope of this manuscript.

3. The authors could not evidence high MW cdc27 complexes in smo/smo transgenic medulloblastoma mouse model. However cdc27 protein levels were lower in curcumin-treated tumors compared to untreated tumors. Are these complexes detectable in DAOY xenograft models (also used in previous publications by the authors) treated with curcumin compared to control? The curcumin concentrations used in in vitro experiments showing high MW cdc27 complexes culminated in 20-40microM: could these concentrations be detected in mice sera?
As requested we tested the Cdc27 levels in DAOY xenografts. Like in Smo/Smo mice Cdc27 levels were reduced in tumor xenografts of curcumin-treated mice when compared to control mice. And as observed in Smo/Smo mice we could not detect high MW Cdc27 in these tumors, probably also due to the lower levels of Cdc27 in *in vivo* tumors. We included this information on page 14 of the revised manuscript. While we did not measure curcumin concentrations in mice sera our previously published studies using Smo/Smo mice and DAOY xenografts clearly showed that therapeutic levels of curcumin can be achieved in mice. In addition, while not as prominent as with concentrations of 20-40 µM, *in vitro* curcumin concentrations as low as 5 µM resulted in Cdc27 crosslinking (Fig. 2B). In addition, we clearly observed that in several cell lines Cdc27 crosslinking increased with time of curcumin treatment (Fig. 4B). Thus, it is possible that even though the curcumin concentrations in mice sera are not as high as the concentrations used in our cell culture studies, the prolonged treatment of tumor-bearing mice over several weeks might result in a cumulative effect of curcumin.

4. A comment should be made about thymidine block on DAOY cells that only partially triggered a cell cycle block used in fig 1A.

We have now included this information on page 9.

5. In fig 2B, are cells synchronized?

The cells in Fig 2B are un-synchronized. We have now included this information in the figure legend.

6. In fig 3A, are cells treated before pull-down with curcumin-coated beads? A faint band appeared in control lane: did control sepharose beads pull down cdc27 (not phosphorylated)?

Untreated cells were used for the pull-down assays. The faint band of unphosphorylated Cdc27 is probably due to non-specific binding to the sepharose beads.

7. Regarding, cdc27 phosphorylation, 2 references could be included in the discussion.

We have included these references as requested on page 20.

**Reviewer: Claude Prigent**

This reviewer did not request any revisions.